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NsG-173-62

INSTITUTE FOR SPACE BIOSCIENCES

FLORIDA STATE UNIVERSITY TALLAHASSEE, FLORIDA

FIRST ANNUAL REPORT

1 November 1962

OUTLINE

HISTORY

The Institute was officially initiated on 1 November 1961. Its support derives predominantly from the National Aeronautics and Space Administration under Grant No. NsG 173-62. The Institute is also supported by grants from the National Science Foundation, The U. S. Public Health Service, The General Foods Corporation, and The Eli Lilly Company. It is an integral part of the Florida State University. The initial program is a continuation of the earlier studies, within the area defined under OBJECTIVES of three faculty members. Included in the present program are new studies of extraterrestrial matrices and environments and newer studies of comparative terrestrial biology.

OBJECTIVES

The objectives of the Institute were originally described as the investigation of processes involved in the origin, evolution, and development of organisms under terrestrial and extraterrestrial conditions. The emphasis is on comparative biochemistry and other aspects of comparative biology in the universe. In particular, investigations are directed toward the organic chemistry which can emerge under a variety of planetary conditions. Valuable information, for example, may be obtainable from planets which, although having no life, cannot be visualized as lacking carbon compounds.

A more specific listing of the objectives and approaches of the Institute and its faculty is:

(a) To define the conditions under which living matter might arise anywhere.

(b) To compare the conditions on any extraterrestrial body with those of (a) and to compare extraterrestrial matter with terrestrial matter, and

(c) To educate graduate students to develop meaningful new researches in space biology from an intellectual foundation which is both specialized and interdisciplinary.

Approaches to objectives (a) and (b) now include:

(1) Studies of abiogenesis in the earthbound laboratory,

(2) Preparation of devices to measure and sample extraterrestrial environments,

(3) Studies of organic chemistry in extreme geological locales (perivolcanic regions, for example),

(4) Theoretical studies of comparative biochemistry,

(5) Study of models of planetary atmospheres and hydrospheres,

(6) Comparative studies of biological development and its initiation and differentiation,

(7) Investigation of cytogenetic mechanisms,

(8) Inflight experiments on the effect of weightlessness,

(9) Studies of paleo-organic chemistry,

(10) Studies of morphology as related to inclusions in meteorites.

PERSONNEL

The personnel of the Institute are:

Name	Highest Degree	Title	Field of Investigation
S. W. Fox	Ph. D.	Professor (and Director)	Molecular Evolution
S. Hess	Ph. D.	Professor	Comparative Meteorology
T. R. Mann	Ph. D.	Visiting Professor	Biochemistry
C. B. Metz	Ph. D.	Professor	Developmental Biology
K. Harada	Ph. D.	Research Associate	Molecular Evolution
T. Hayakawa	University Diploma	Research Associate	Polymer Chemistry
C. L. Mann	Ph. D.	Visiting Investigator	Developmental Biology
M. Menzel	Ph. D.	Guest Investigator	Cytogenetics
C. A. Shivers	Ph. D.	Visiting Investigator (U.S.P.H.S. Post-doctoral Fellow)	Developmental Biology
P. Babcock	D. D. S.	Research Assistant	Developmental Biology
E. Bradley	M. S.	Research Assistant	Chemistry
R. Cheng	B. S.	Research Assistant	Meteorology
T. Fukushima	B. S.	Research Assistant	Developmental Biology
D. Hampson	B. S.	Research Assistant	Chemistry
E. Wiggert	M. S.	Research Assistant	Chemistry
C. Windsor	B. S.	Laboratory Assistant	Chemistry
R. T. Brown	B. S.	Graduate Research Assistant	Meteorology
L. Franklin	B. S.	Graduate Research Assistant	Developmental Biology
C. Genaux	M. S.	Graduate Research Assistant	Chemistry
A. Gruber	B. S.	Graduate Research Assistant	Meteorology
R. Hadlock	M. S.	Graduate Student (U.S. Steel Pre-doctoral Fellow)	Meteorology

Name	Highest Degree	Title	Field of Investigation
P. D. Hoagland	M. S.	Graduate Research Assistant	Chemistry
D. Rohlfing	M. S.	Graduate Student (U.S.P.H.S. Pre-doctoral Fellow)	Chemistry
L. Rustad	B. S.	Graduate Student	Developmental Biology
A. Schwartz	M. S.	Graduate Research Assistant	Chemistry
S. Stern	M. S.	Graduate Research Assistant	Developmental Biology
K. Stewart	B. S.	Graduate Student (U.S.P.H.S. Pre-doctoral Fellow)	Chemistry
J. Bronson		Student Assistant	
C. Darby		Student Assistant	
D. Joseph		Student Assistant	
T. Limbach		Student Assistant	
R. McCauley		Student Assistant	
S. Steen		Student Assistant	
V. Bowser		Secretary	
J. Cox		Secretary	
M. Dockendorf		Executive Officer	
M. Franklin		Executive Secretary	
J. Goldinger		Curator-Stockroom Assistant	
H. Hendry		Expeditor and Accountant	
L. Roddenberry		Adminstrative Assistant (half time)	
G. Smith		Machinist's Assistant	
N. Smith		Machinist	
S. Sportelli		Stockroom Clerk	
R. Williams, Jr.		Machinist's Assistant	

WORKING SPACE

At the present time the Institute for Space Biosciences occupies part of the second floor of the Conradi building, some of the ground floor and fourth floors of the Mathematics-Meteorology building, and part of the fifth floor of the Molecular Biophysics building. Approximately 3,000 square feet of net working space have been made available to the faculty of the Institute since its formation, although not on a permanent basis.

PROGRESS IN RESEARCH

Experimental Studies of Abiogenesis

Stages in abiogenesis can be conveniently divided into:

1. Proliferation of micromolecules.
2. Generation of macromolecules.
3. Origin of metabolism.
4. Precellular organization.
5. Generation of cells.

Experimental results interpretable in each of the first four contexts will be discussed. These are results and summations obtained mostly within the year of record.

Proliferation of Micromolecules (Fox, Stewart)

Mr. Kent Stewart has studied the thermal reaction of formose (the sugary formaldehyde condensation product) with urea to give at least ten amino acids. The origin of many individual amino acids had earlier been explained. For some, no experimental demonstration has been advanced. Most needed is a system which generates simultaneously all of the proteinaceous amino acids.

Dr. K. Bahadur (with the Institute since December, 1962) reported in 1954 (Nature, 173, 1141) the simultaneous synthesis of nine amino acids by ultraviolet radiation of formaldehyde and a solution of urea.

Generation of macromolecules (Fox, Harada, Rohlfing, Hoagland, Genaux)

Further characterization of thermal proteinoids was reported during the year.

In particular, Mr. D. L. Rohlfing has observed that the catalytic activity of proteinoids for p-nitrophenyl acetate is destroyed by heating them in aqueous solution at pH 6.8 in a boiling water bath for 5-20 minutes. Dr. Gottfried Krampitz at the University of Bonn has observed that the hydrolysis of proteinoids by pepsin is greatly increased, and that by trypsin increased under the same conditions. He had earlier studied the effect of 8 M urea, which behaves in a way similar to that of wet heat. Rohlfing's results were reported at the

meeting of the American Chemical Society in Washington, D. C. in March 1962.

These results demonstrate that heating of solutions can destroy catalytic activity of macromolecules prepared by heating of dry amino acids (with sufficient aspartic acid in the mixture). This is of particular interest in view of the unqualified preconception that polyamino acids closely resembling proteins could not be produced by heat, since heat is a well-known agent for denaturation. The critical condition, of course, is whether the heat is applied to the dry or wet state.

Dr. K. Harada has developed methods for quantitating C-terminal amino acids, free carboxl groups, and dicarboxylic amino acid residue ω- amides. The evidence for the peptide bonds in proteinoids now includes biuret test, 100% hydrolysis to amino acids by mineral acid, infrared absorption peaks at 3300, 3080, 1650, and 1550 $cm.^{-1}$, and hydrolyzability by proteolytic enzymes. These are the chief criteria with which the peptide structure of proteins was established (see Fox and Foster, Introduction to Protein Chemistry, John Wiley and Sons, Inc., 1957).

More detailed information about the backbone structure has been obtained from analyses of hydrolyzates, isolation of peptides by partial hydrolyzates of thermal copolyamino acids, and C-terminal analyses.

Studies of the hydrolysis and analysis of proteinoids, partly in collaboration with Dr. Kenneth R. Woods of the Cornell University Medical College, have been completed. Much of this work was done by Dr. K. Harada and Mr. C. R. Windsor on an amino acid analyzer, Fig. 1, purchased with NASA funds. Analyses have revealed much internal control, during the synthesis, of amino acid contents of proteinoids. Such studies show also that crude proteinoids contain nonpolyamino acid material (also observed at the Ames Research Center) and that these preparations can be purified to 100% polyamino acid. In order to recover amino acids 100%, Fig. 2, hydrolytic periods longer than typically used on proteins are necessary. This is consistent with the inference from structural studies that proteinoids are comparable, in degree of complexity in their internal structure, to that of proteins.

Mr. Charles Genaux reported at the American Chemical Society meeting at Atlantic City in September on the participation of cystine in structure of thermal polyamino acids. Because of some uncertainty about cystine on analyzer

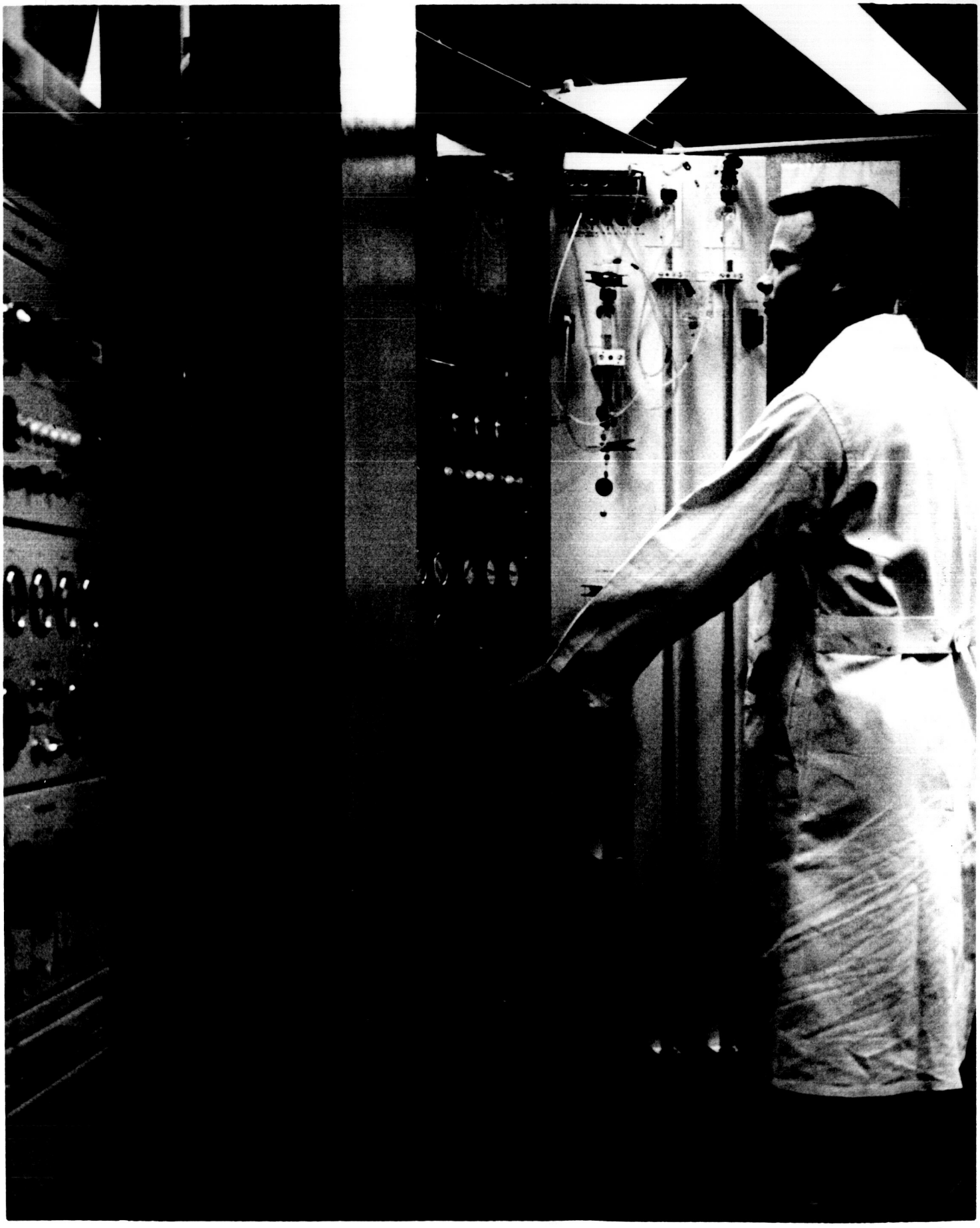

Fig. I. Mr. C. R. Windsor operating an amino acid analyzer.

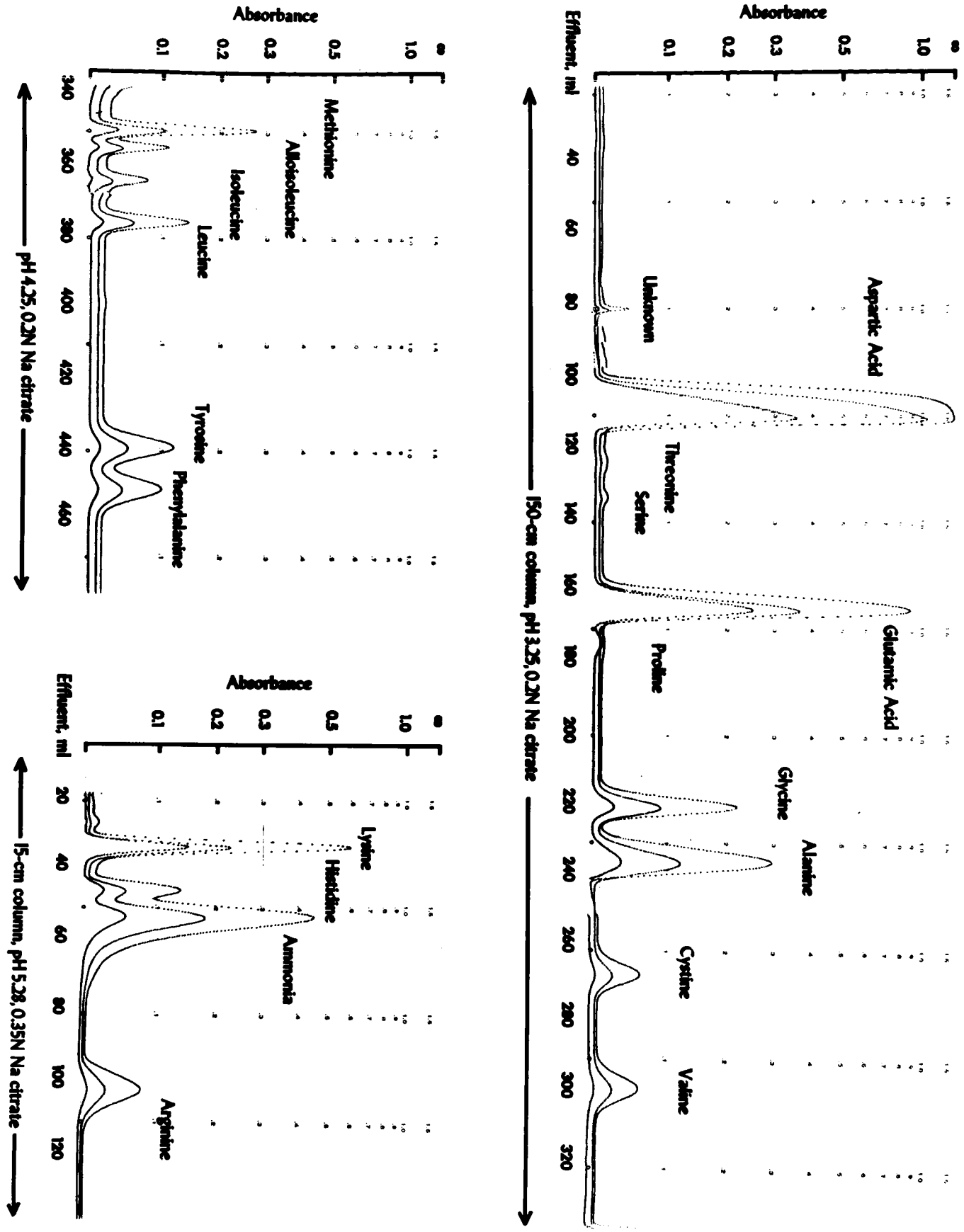

Fig. 2. Analyzer chromatogram of a 2:2:3 proteinoid.

Fig. 3a. Electron micrographs of osmic acid-stained sections of proteinoid microspheres obtained from methacrylate blocks.

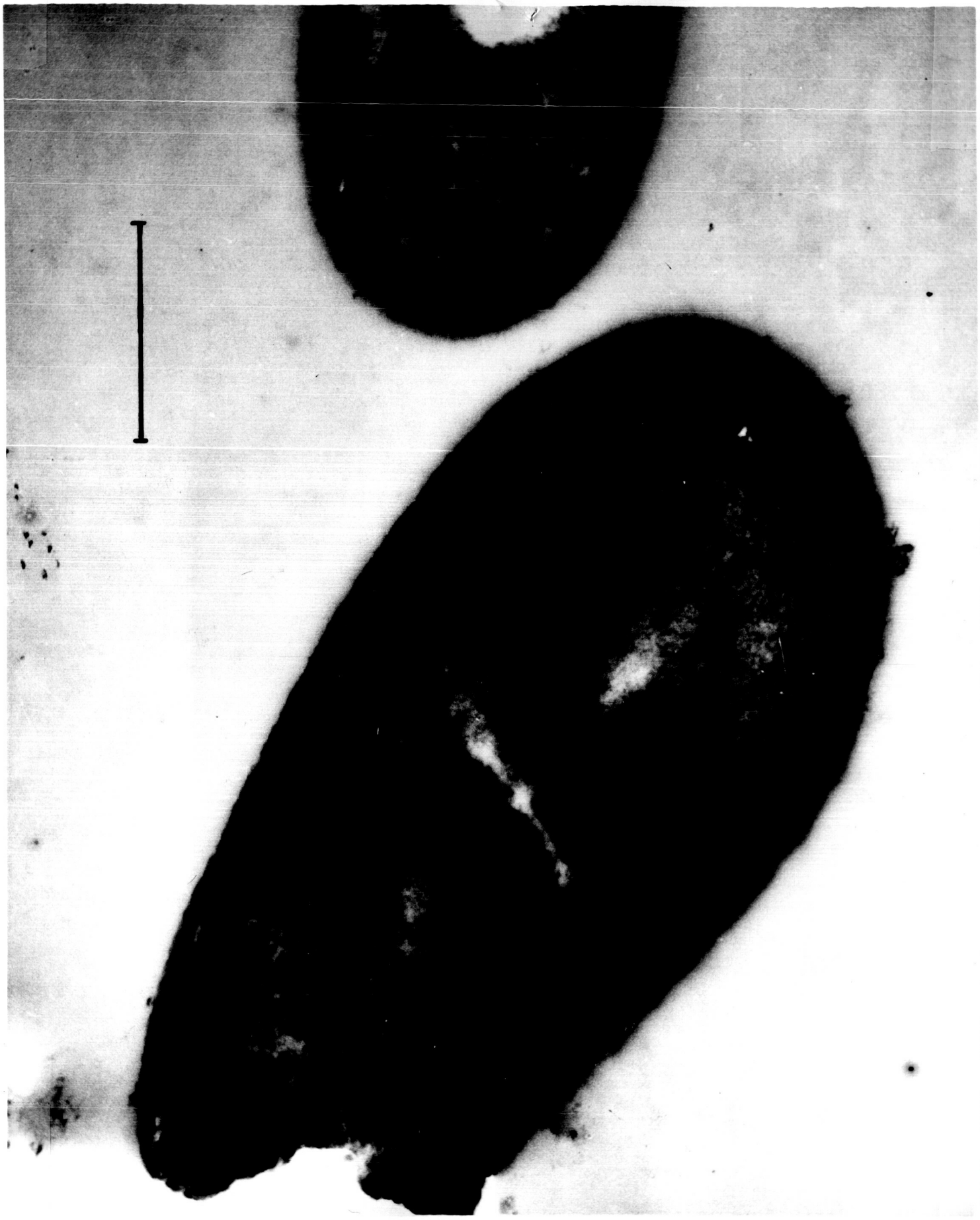

Fig. 3b. Electron micrographs of osmic acid-stained sections of proteinoid microspheres obtained from methacrylate blocks.

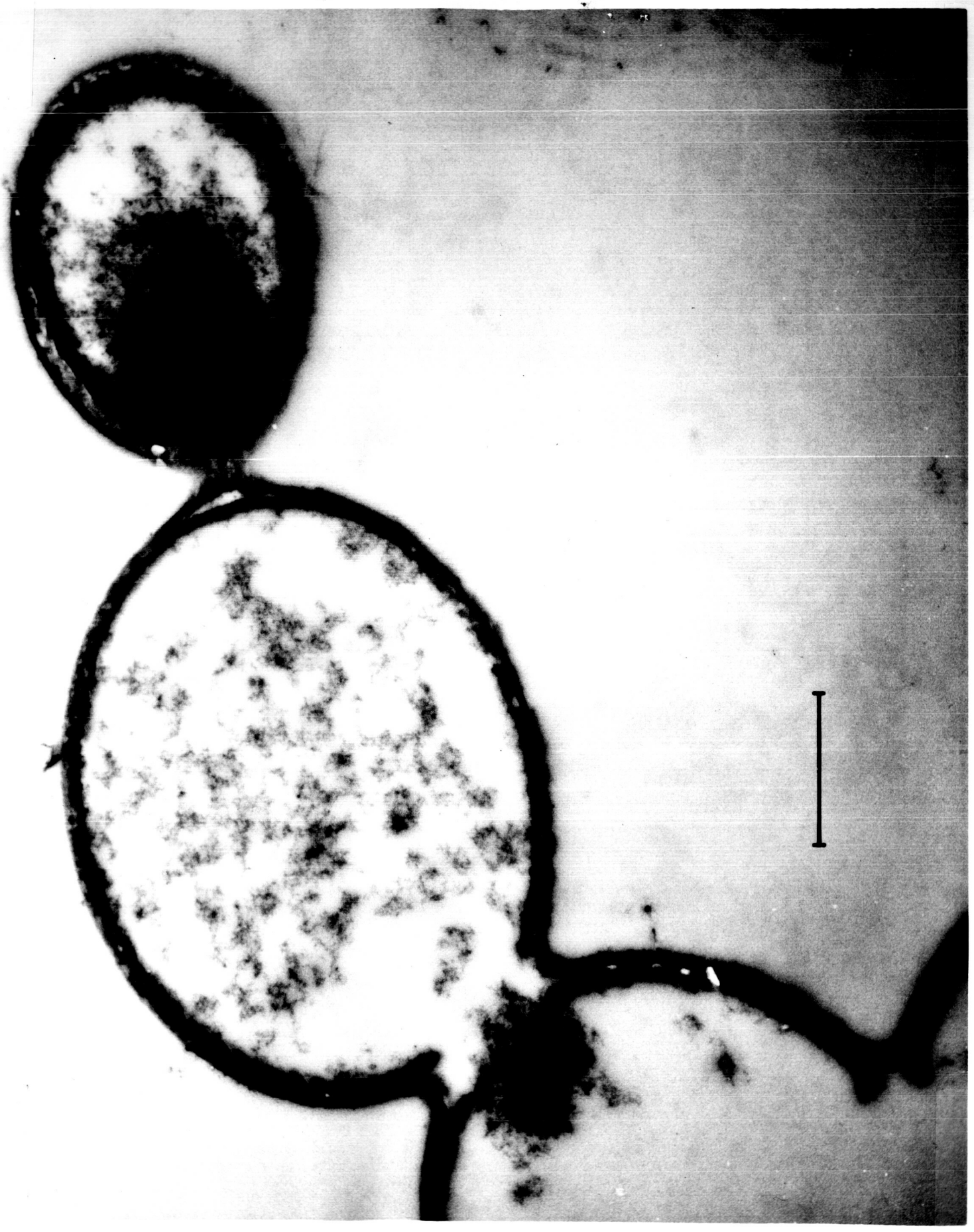

Fig. 4a. Same as 3a and 3b with microspheres which have first, however, been subjected to elevated pH.

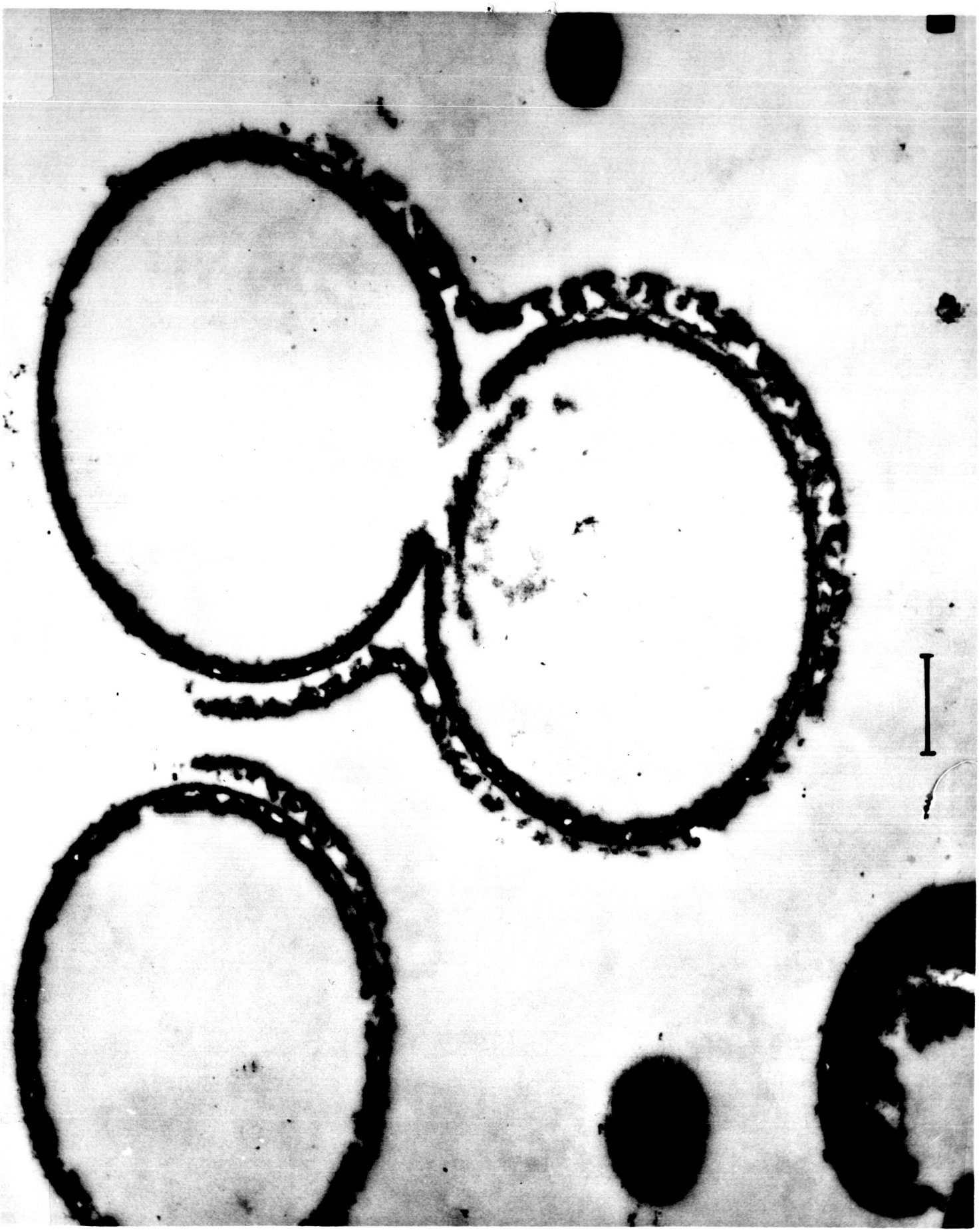

Fig. 4b. Same as 3a and 3b with microspheres which have first, however, been subjected to elevated pH.

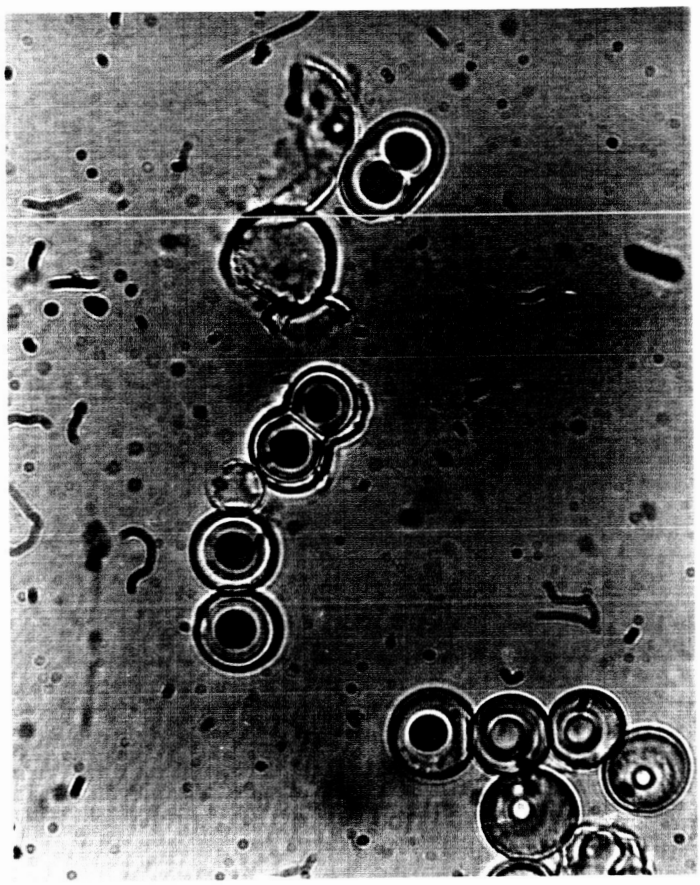

Fig. 5. Twinned microspheres obtained by S. Yuyama.

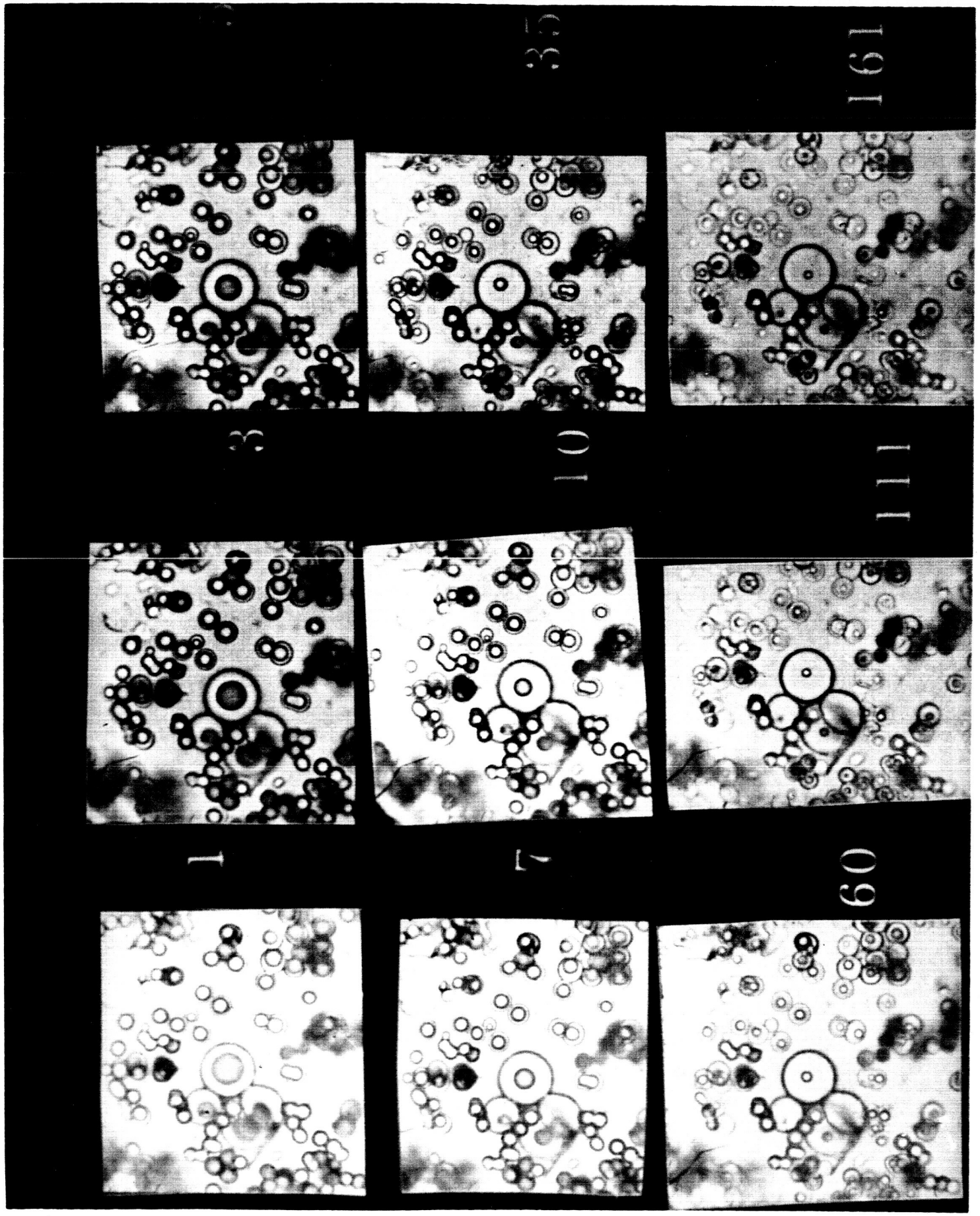

Fig. 6. Time lapse sequence of proteinoid microspheres with elevation of pH. Time between No. 1 and No. 161 is 80 minutes, between No. 1 and No. 35, 17 minutes, etc. In the center may be seen a microsphere in which the center disappears while the boundary remains. This field contains several examples of microspheres showing separation of two contained centers. (S. Yuyama and R. McCauley)

Fig. 7a.
Gram-negative microspheres.
Units are pink with counter-stain of Safranin 0.

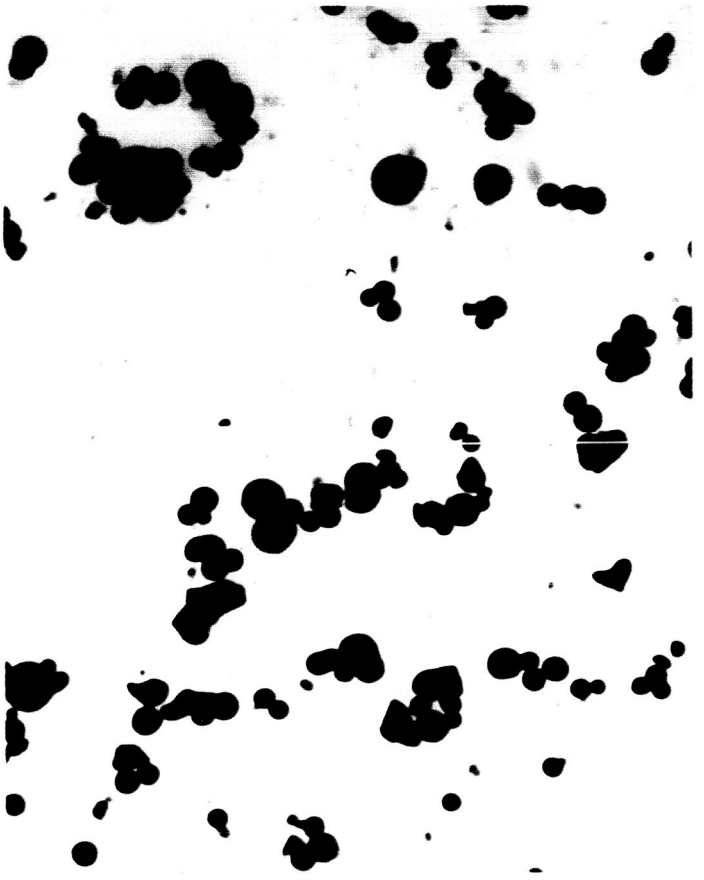

Fig. 7b.
Gram-positive microspheres.
Units are deep purple.

Fig. 8. Precise machining of the large shaft for the rotation appartus is one step toward the completion of an experimental system that will provide simulation of planetary atmospheres with a view toward more complete understanding of the dynamics and general circulation of these atmospheres.

Fig. 9. An experimental model of the Frost Point Apparatus with simulating and data recording systems for determining the water vapor in the atmosphere of Mars.

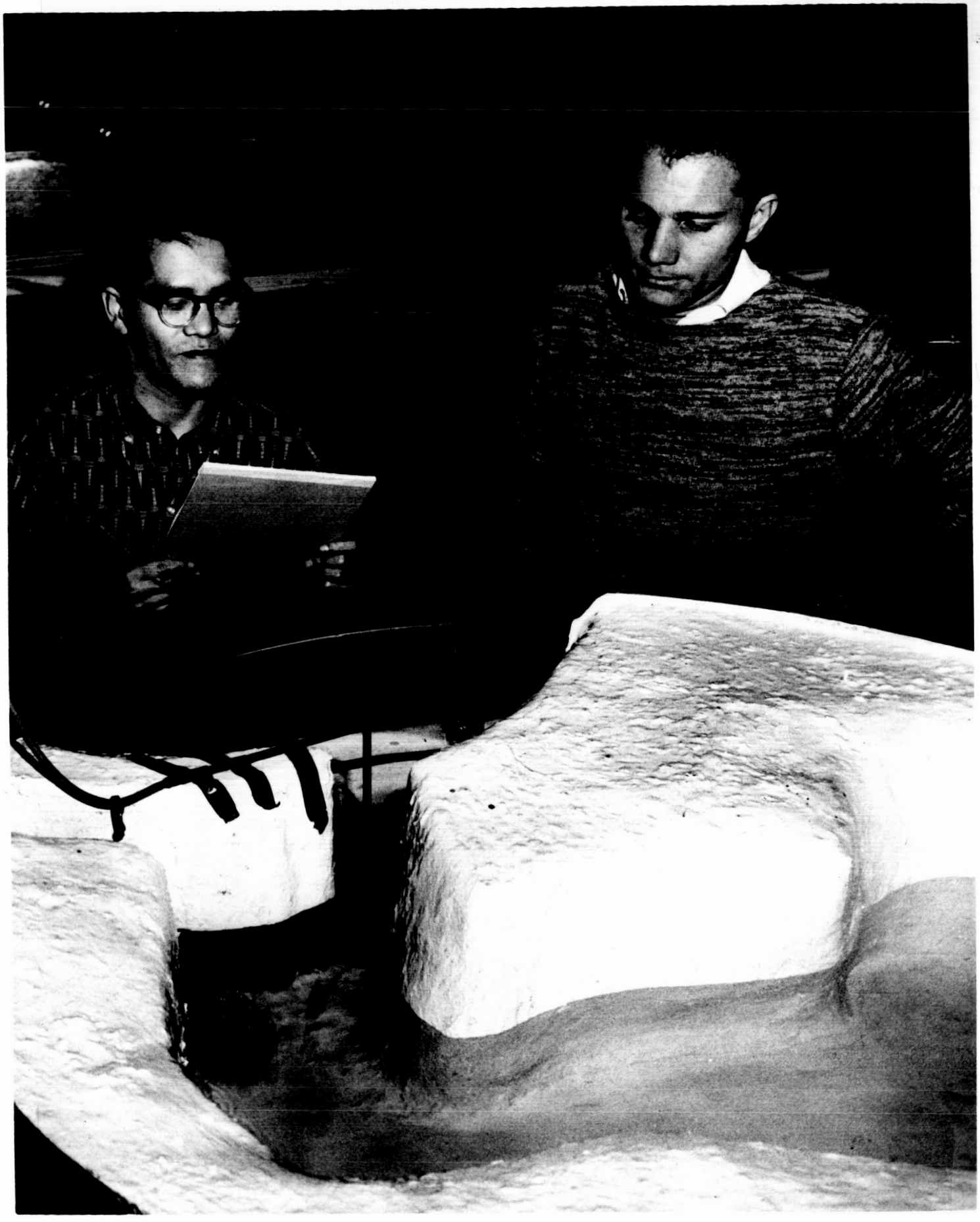

Fig. 10. A rotating model experiment on the ocean current through the Yucatan Channel in the Gulf of Mexico. (Dr. Ichiye and Mr. Plutchak)

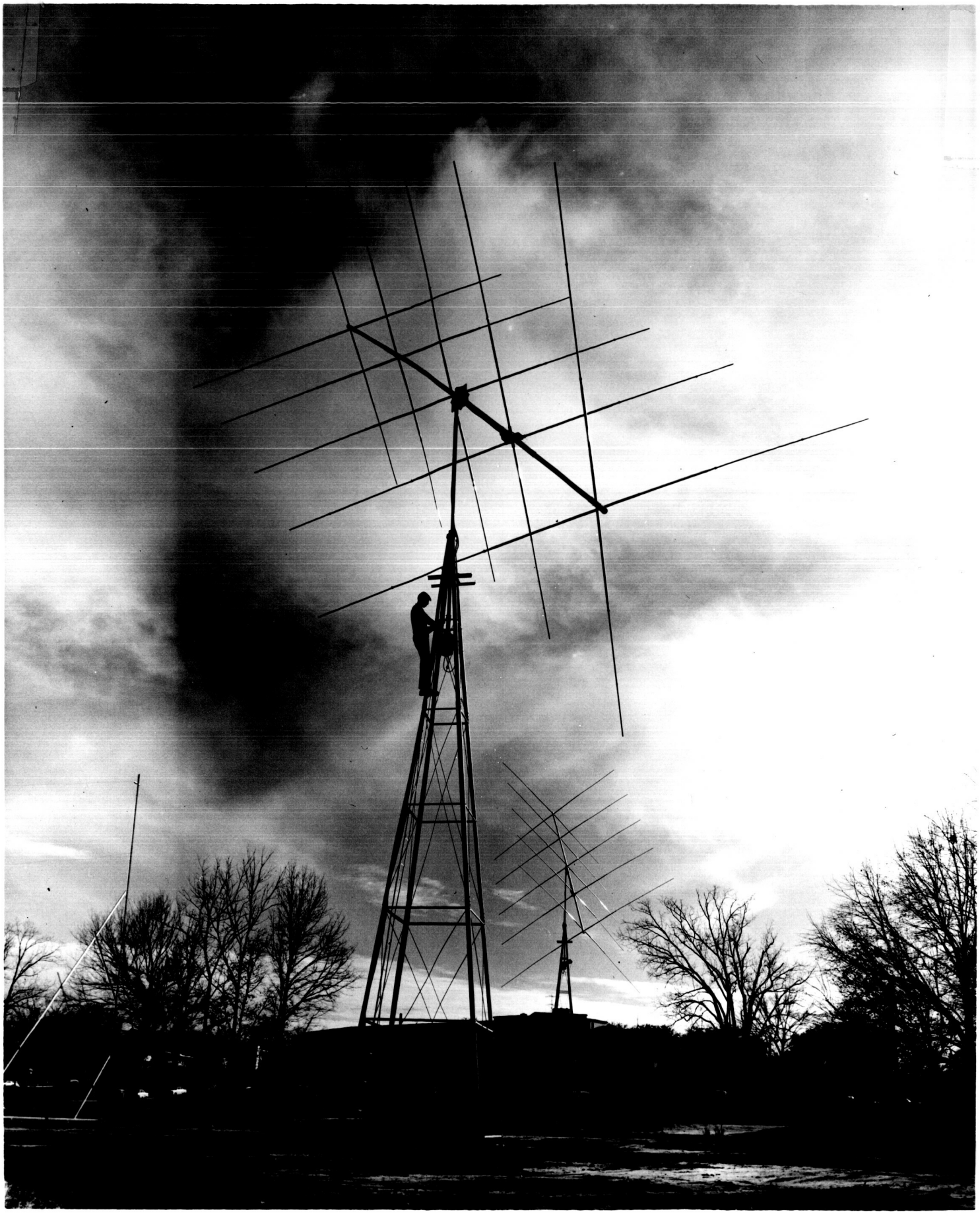

Fig. 11. One of the Radio Polarimeters of the Florida State University radio observatory. (Professor Colin Barrow)

chromatograms, Mr. Genaux has oxidized cystine residues with performic acid and analyzed for cysteic acid. These values are mostly quantitatively equivalent to the cystine assayed in the hydrolyzates of the unoxidized material. When these polymers (from aspartic acid, glutamic acid, alanine and cystine) are either oxidized with performate or reduced with mercaptoethanol, the product shows a rate of diffusion through Craig membranes approximately twice as great as in the original. The results suggest the presence of cross-linked cystine, as in insulin.

Studies of the nutritional quality of proteinoids for mammals are being carried out with Drs. G. Krampitz and F. Knappen of the University of Bonn, using first a special proteinoid prepared by Dr. Harold A. Campbell, research fellow of The General Foods Corporation. Other proteinoids for this purpose have been more recently prepared by Mr. David Joseph working under Dr. Harada's supervision. One publication in this area by Drs. Krampitz and Knappen is to be found in Nature 195, 385 (1962).

Krampitz has extended the findings of this laboratory on the substrate behavior of proteinoids as acted upon by mammalian proteases. Of particular interest is his unpublished finding (citation by permission) that the proteolyzability of proteinoids is greatly enhanced by treatment with 8 M urea or heating in aqueous solution.

In an overall sense, a salient feature is that one can produce by dry heat macromolecular catalysts which are inactivated by wet heat.

By use of a Titrigraph, Mr. P. D. Hoagland is finding that some special structural features of proteinoids, particularly imide linkages, can be studied.

In the context of abiogenesis, and of peptide and protein chemistry, salient contributions from these studies as viewed at this time, are:

1. Amino acids can be copolymerized by heat into a wide variety of

heteropolyamino acids, under suitable conditions.

2. All of the eighteen amino acids common to protein can be simultaneously copolymerized thermally. Although two of these (serine, threonine) suffer substantial decomposition under the usual conditions employed, some of each of the amino acids in the reaction mixture are found in the product.

3. The 18 - amino acid polymers, known as proteinoids, have many properties in common with proteins.

4. Although the essential reaction is rugged and methodologically simple, the products are complex and delicately structured.

5. Study of hundreds of syntheses of proteinoids indicate that the necessary conditions are sufficiently wide and have probably been so widespread that these processes have the quality of inexorability in a cosmic sense. Chemists tend to visualize simple processes for simple substances, complex processes for complex substances. These results show how complex macromolecules e.g., proteins, could have simply arisen in many places at many times.

Mr. Tadao Hayakawa has prepared proteinoids through the Leuchs method. This synthesis has proved to be intricate. Studies of availability of all "essential" amino acids from thermal and Leuchs proteinoids can now be undertaken and this kind of study was emphasized as a coming area in nutritional research by Dr. Richard Barnes at the Symposium on Protein Nutrition and Metabolism at the University of Illinois in October, 1962.

Studies of the effect of proteinoids on natural substrates are continuing and are being intensified, particularly by Dr. Bahadur and Mrs. Elizabeth Wiggert. Mr. Hayakawa has continued studies seeking to produce peptides analogous to active sites of enzymes.

Thermal Synthesis of Polynucleotides (Fox, Schwartz, Bradley)

The fact that molecules as complex as proteins can be generated by a simple method has raised the question of a similarly simple route to polynucleotides. This possibility has been under investigation in this laboratory for many years. G. Schramm has recently claimed that he has thermally polymerized mononucleotides

in the presence of ethyl polyphosphate, under conditions identical to those used here to polymerize amino acids. Ethyl polyphosphate is neither easily prepared nor a likely prebiological compound, but Schramm's report has suggested new experiments with polyphosphoric acid.

Polymers from cytidylic acid have been prepared in this laboratory in various ways by use of the free polyphosphoric acid at 65°, including with urea. The product contains both higher polyphosphoric acid and some kind of polymer from cytidylic acid. This polymer shows hyperchromicity, high molecular weight, u. v. absorption typical for cytosine, and the typical shift in u. v. on treatment with alkali.

Production and characterization of microspheres

(Fox, Yuyama, Fukushima, Kendrick, McCauley)

If proteinoids are dissolved in hot aqueous solution which is allowed to cool for a few minutes, huge numbers of microspheres in the shape and range of size of the cocci separate from the clear solution. These microspheres received major new attention during the year. These units are alternative to the precellular model of Oparin - the coacervate droplet. Oparin has commented in his books on the lack of stability in the coacervate droplet, and this is one of many respects in which the proteinoid microsphere is of more interest as a model antecedent of the cell. The microspheres may also be sectioned, and are relatively uniform in size. In each of these respects they are distinguished from coacervate droplets.

Centrifugeability of microspheres

The microspheres are typically spun in a clinical centrifuge at some stage of their preparation. No noticeable change results, much as is the case with bacteria similarly treated. The coacervate droplets coalesce into two layers under such treatment.

Sectionability (Fox, Fukushima)

Drs. Richard S. Young and J. Miguel with E. Munoz at the Ames Research Center discovered during the year that microspheres could be set up in paraffin blocks and sectioned by microtome. This finding has been extended in the

Institute. Methacrylate blocks may be used, and sections of 800 A^{o} thickness prepared.

Electron Micrography (Fox, Fukushima)

With the aid of Dr. Charles Metz and Mr. Luther Franklin, equipment bought with NASA funds (LKB Ultratome) and Mrs. C. Dockery of the Department of Biological Sciences, electron micrographs were prepared of microspheres. In Figs. 3a and 3b are seen typical sections of microspheres. The granular constitution resembles the granular cytoplasm seen in bacterial cells as depicted by Murray (I. C. Gunsalus and R. Y. Stanier, The Bacteria, Vol. I, Academic Press, 1960, p. 35). Not much detail is observed in micrographs of bacterial sections as compared to cells of higher organisms. This lack of detailed structure in some bacterial cells may however be significant in an evolutionary sense.

In Figs. 4a and 4b are seen electron micrographs of derivatives of microspheres which have been transferred to solution of higher pH. Notable is the indication of a double membrane structure. These structures were produced in the absence of added lipid. The only lipid-like material that might be present would be a residue from alcohol washing of the proteinoid, or the hydrocarbon side chains of the amino acid residues of proteinoid themselves.

Twinning

The proteinoid shows considerable tendency to form microspheres. This tendency is not limited to the cooling of hot solutions. Dr. Young (Ames Research Center) reports that chilling of a solution at room temperature gives nicely formed microspheres. In this laboratory it has been found that microspheres can form from proteinoid at room temperature when treated with water.

This considerable tendency for the macromolecular mass to form microspheres (in the size and shape of the cocci) is ramified by a considerable tendency of those to form twinned microspheres. Such a field is shown in Fig. 5. This picture resembles one which has appeared in the Life-Time

book, "The Earth", over an inaccurate caption. Such behavior is of interest particularly as indicative of the inherent tendency of appropriate macromolecules to assume, under appropriate conditions, forms which characterize cellular units at salient stages in the dynamic processes in which they participate. In the case of Fig. 5, it is not possible, from such pictures alone, to determine whether the structures shown are the result of fusion, cleavage, or of the occurrence of this shape at the moment of formation.

A partial answer to this question is given by Fig. 6, which includes nine frames from a time-lapse sequence. Frame no. n + 1 follows frame no. n by 30 sec.. Accordingly, the last frame, no 161, is 80 min. later than no. 1. The field shown consists of microspheres which were prepared in water (pH about 3.0) and the pH subsequently raised to 5.5-6.5 by addition of phosphate-citrate buffer. Separation of centers can be seen in several microspheres followed through the sequences. The exact meaning of this phenomenon is under intensive investigation, and will probably not be clearly interpretable until, and if, coupled with other phenomena being studied.

Integrity of boundaries

Fig. 6 also shows how the membrane, or boundary, of the microsphere differs in behavior from the rest of the microsphere. This is evident from the progressive change in the larger unit in the center of each frame.

Workable quantities of the membranes have been prepared and are being analyzed in the effort to determine what material attribute distinguishes the boundary from the remainder.

Diffusion (Fox, Fukushima)

Microspheres were made in 2% solution of each of four saccharides and subsequently washed four times with water. The monosaccharides, glucose and fructose, were entirely lost by washing, but the microspheres prepared in solutions of each of the two macromolecular carbohydrates, glycogen and starch, retained significant proportions of these.

Other tests are being applied to determine if the selectivity is one

dependent upon selective diffusibility of small molecules contrasted to that of large molecules, or whether attractive forces for large molecules may be responsible.

Gram Stain (Fox, Yuyama)

Although uniform microspheres can be easily produced from acid proteinoid, they are not produced from the basic lysine proteinoid. Microspheres can however be produced from mixtures of the acidic and basic type, if the acidic type is the major part of the mixture. When the basic proteinoid in the mixture is more than 35 - 40%, the microspheres stain Gram - positive. When the proportion is less, they stain Gram - negative.

These results agree with indications in a decades - old literature on the theory of the Gram stain, (a) to the affect that the stain is for proteins which make up the bacterial cell, and also agree with (b) newer suggestions that Gram - positiveness requires sufficient basic material in the cell (Fig. 7). A detailed publication is in press in the Journal of Bacteriology (preprint has been circulated).

Relationship of Microspheres to Formed Elements in Meteorites (Fox)

Many of the figures published by Claus and Nagy as representative of formed elements in meteorites resemble in size and shape microspheres and microsphere derivatives seen in this laboratory in experiments having other purposes. This relationship is presented in detail in a paper in press in the Annals of the New York Academy of Science (preprint has been circulated).

One may infer that the units observed in meteorites, if not terrestrial contaminants, were (1) once alive, (2) are merely physiochemical phenomena of a special type or are (3) prebiological evolutionary experiments. Possibility (2) is in a sense a type of (3). If these are prebionta rather than protobionta, they may be of greater interest as the former. These conclusions were presented orally at the AAAS meeting in Denver, Colorado on 26 December 1961 and at the New York Academy meeting on 1 May 1962. With reference to the same data, Philip Morrison published the same conclusions in

Science (1962) and these were abstracted by H. Urey in Science, 137, 623 (1962).

Extraterrestrial Macromolecular Sampler (Fox, Hobby, Windsor)

With Mr. George Hobby as the cognizant scientist at JPL, and with interest expressed by Beckmann Instruments Co., work has begun on a method and device for sampling macromolecules and telemetering data back to earth.

A 4 hr. hydrolysis sample of 2:2:3 proteinoid, used as a model of an extraterrestrial polyamino acid, has been found to give a profile very much like that of a 48 hr. hydrolyzate with however lesser values for each amino acid.

Sampling of Extreme Environments (Fox, Harada)

Borings at Stromboli to a 3 foot depth (temp. 92^{o}) were made, and some analyses have been performed. Indication of several organic nitrogen compounds was found. Further analyses of samples on hand may help answer the question of whether these compounds are the result of contamination rather than the product of perivolcanic processes. If not, the sampling may have to be repeated and extended under aseptic conditions. Samples of early strata in Montana have been obtained for similar purposes.

Planetary Atmospheres

Theoretical studies (Hess, Joern)

The theoretical investigation by Hess and Joern of the vertical structure of the Venus atmosphere has progressed to the point where computer programs have been written and checked, and several thousands of individual numerical results have been obtained. These are in the form of combined theoretical-observational optical thicknesses for various layers of the Venus atmosphere under several assumptions as to composition.

With the departure of Capt. Joern to his next military assignment, the bulk of the computing work has been shifted from Florida State University to his present location at Offutt Air Force Base, Omaha. Joern is using computing facilities at Offutt AFB to continue the project. The first numerical results dealing with vertical temperature distributions are expected in the near future.

Model atmospheres (Hess)

The machine shop is now devoting almost all its efforts to construction of the rotating apparatus (Fig. 8) for model investigations of the atmosphere of Jupiter. Considerable material and equipment needed for this major effort have arrived or are expected very shortly. Actual experimental work should begin early in February, 1963.

The laboratory model of apparatus (Fig. 9) designed to measure extremely low amounts of water vapor in the atmosphere of Mars has been rebuilt completely to take advantage of experience gained with the first version. Work with this new model has recently begun.

Genetic Mechanisms

Plant tissue culture studies (Menzel)

Work with undifferentiated plant callus tissue in sterile culture was initiated with two objectives in mind: (1) To establish such a system in a form suitable for studying the structure and behavior of chromosomes and their reactions to mutagenic agents, and (2) to determine whether such a system could be utilized to insert into the life cycle of higher plants a single - or few-celled stage which would make such complex biological species more accessable to artificial evolutionary mechanisms. Haplopappus garcilis, a small composite native to the Great Plains, was chosen as the test organism for the initial stages of investigation because it has only two (readily distinguished) pairs of chromosomes.

Most of the equipment and materiel necessary to initiating this work have now been received, and it has been determined that callus growth can be initiated on seedling hypocotyls and maintained through at least nine transfers on a completely defined medium consisting of agar, minerals, sugar and vitamins (Murasnige's tobacco medium). Two callus clones were selected from about 25 original clones because they gave vigorous and fairly uniform growth in subculture. Unfortunately, one of these clones proved to be tetraploid and the other a mixture of diploid and tetraploid cells. Diploid strains would be more desirable and efforts to obtain them are in progress.

The mitotic index in the two selected strains is very low under the standard conditions of culture. Present efforts are concentrated upon testing the effect of addition to the medium of various plant growth factors in the hope of increasing the rate of cell division to a more workable level.

Evolutionary divergence of chromosomes (Menzel)

Accumulation of evidence in recent years has led to the suspicion that mutation and linear rearrangements within chromosomes are insufficient to explain chromosome evolution at the level at which formerly homologous chromosomes exhibit the stigmata of partial non-nomology (failure of synapsis and / or failure of chiasma formation). The hypothesis has been advanced that a change in the timing of one or more stages of the meiotic chromosome sequence may be involved. Taylor and others have recently demonstrated that one such category of partially nonnomologous chromosomes, namely sex chromosome pairs, undergo DNA replication at different times in the nuclear cycle. Research is being initiated to determine whether the same may be true of partially nonnomologous chromosomes in interspecific hybrids. Preliminary work in progress involves acquiring equipment and supplies necessary for making autoradiographs, developing a technique for labelling meiotic prophase chromosomes with H^3 - thymidine, and screening various interspecific hybrids for suitability.

Studies on Fertilization Physiology

Inhibitors of fertilization (Metz, Shivers)

Agents (fertilizin, antifertilizin) from eggs and sperm are known to inhibit fertilization of sea urchin eggs under certain conditions. These substances act by blocking the complementary cell surface substances. Such action implies that the cell substances in question have a role in fertilization. This role probably involved attachment of the sperm to the egg.

Likewise certain agents from the integument of the sea urchin, Arbacia and the alga, Fucus, also inhibit fertilization in sea urchins and other organisms. These agents act by blocking some essential egg surface substance that appears

to be unrelated to the fertilizin-antifertilizin system. The egg substance(s) in question appears to be localized in the egg surface, to be removed or destroyed by proteolytic enzymes and to be associated in some way with the block to polyspermy. Additional experiments will attempt a further clarification of the nature, location and specific role in fertilization of these substances using the above inhibitors.

A third class of fertilization inhibitors includes specific antibodies prepared by immunizing rabbits (or other animals) with sea urchin gametes or appropriate extracts. As first shown by Tyler (1946) appropriate antibodies inhibit fertilization by combining with specific sperm or egg antigens. Extensive mapping studies of the sea urchin sperm surface using absorption techniques have recently been carried out in this laboratory. The studies show that the sperm surface contains both soluble and insoluble antigens. The latter are apparently confined to the sperm head region. Absorption experiments using isolated sperm heads, tails and heterologous sperm have demonstrated additional antigens. Localization of the antigens is being studied by the use of the fluorescein labelled antibody technique of Coons and the ferritin labelled antibody technique in combination with electron microscopy. Experiments in progress show that treatment of sperm with antibody, rendered univalent by digestion with papain, results in a loss of sperm fertilizing capacity. Absorption experiments demonstrate that only one of three antigens could be involved in such inhibition. It is anticipated that this line of investigation may result in localization of new sperm surface substances and elucidation of their role in fertilization. Two additional problems of considerable current interest are under investigation. The first of these concerns the mechanism of the acrosomal reaction. This conversion of the normally compact sperm acrosome into an extended filament was first described by J. C. Dan in 1952. Since that time several investigators have examined the morphology of the unreacted and the reacted acrosome with electron microscopy and related the acrosomal reaction to morphological aspects of fertilization. However, no thorough study of the mechanism of the reaction has been done. The

various means for inducing the reaction include treatment of sperm with supernatants from egg suspensions. This implies that the reaction can be initiated by a diffusible egg substance, possibly fertilizin. Characterization and identification of the active agent in egg supernatants has begun and specificity relationships are under investigation. In recent experiments one anti-sperm rabbit serum was found by Dr. Shivers to initiate the acrosomal reaction. This opens interesting possibilities for identifying an acrosomal reaction trigger on or in the spermatozoan.

Fate of sperm surface membrane at fertilization (Metz, Austin)

According to views held until recently, the entire sperm head and midpiece, including the limiting membrane was thought to enter the egg. However, recent electron microscope evidence from several laboratories indicates that at least in some forms the sperm surface membrane coalesces with and becomes a part of the egg membrane. Our studies involve marking the sperm surface membrane with ferritin conjugated antisperm antibody and examining the egg surface for the ferritin (electron microscopically).

Work of associates of Metz

The projects described above relate directly to the activities of Metz and his students and assistants. Others who will be working in Metz's laboratory during 1962-63 on associated problems include Dr. C. A. Shivers, U. S. Public Health Service Postdoctoral Fellow (1961-63), Dr. Thaddeus Mann, F. R. S., Director of the Agricultural Research Council Unit of Reproductive Physiology and Biochemistry, and Reader of Physiology of Animal Reproduction in the University of Cambridge, Cambridge, England, and Dr. C. Lutwak-Mann of the same university.

Dr. Shivers' work is concerned primarily with immunochemical aspects of fertilization in frogs. He has localized specific antigenic components in frog egg jellies as well as other components that are common to other species as well. Treatment of the eggs with antijelly sera prepared in rabbits inhibits fertilizability of the eggs. This effect is obtained by use of antisera rendered univalent by digestion with papain. It seems clear

then that inhibition of fertilization in the frog by anti egg jelly sera results from blocking of some component or components in the jelly that perform an essential role in fertilization. Additional experiments will be directed toward determining which of the several egg jelly antigens are essential for fertilization. This will be done using papain digested antibody that has been absorbed with egg jelly preparations from other species.

Some evidence has accumulated that antigenic substances of the frog egg surface have essential functions in fertilization. Additional studies of such non-jelly antigens will be made using antisera prepared against ovarian eggs.

In the sea urchin, Tyler and Brookbank have shown that anti-jelly sera inhibit cleavage. Dr. Shivers plans a thorough search for similar effects in the frog using anti sera prepared against frog egg jelly, egg supernatants and coelomic eggs.

During the "off seasons" when frog material is not available, Dr. Shivers has been studying the acrosomal reaction of sea urchin sperm. He has discovered that acridine orange treated sperm undergo the acrosomal reaction when illuminated. Thus it is possible to observe the acrosomal reaction at will as it takes place under the microscope. Admittedly this is a highly artificial system, but one which may nevertheless give valuable information about the reaction.

Dr. and Mrs. Mann will continue some of studies on biochemistry of mammalian gametes and early development in our laboratory during 1962-63. In addition, they will investigate some of the local marine invertebrate material for suitability to their research interests.

Inflight experiments (Metz)

Metz's inflight experiments are conducted in collaboration with Dr. R. S. Young of the Ames Research Laboratory. Three attempts have so far been made to examine for an effect of weightlessness on fertilization of sea urchin eggs. In the most recent experiments (Bios I) an attempt was also made to determine if weightlessness affected cell division (cleavage of

sea urchin eggs). Unfortunately these inflight experiments so far have not been successful due to technical failures. These and related inflight experiments will be continued in collaboration with Dr. Young as vehicular opportunities become available.

Fertilization and Gamete Physiology Training Program (Metz)

During the summer of 1962, Dr. Metz directed a training program of the above title at the Marine Biological Laboratory, Woods Hole, Massachusetts. This program is supported by a training grant from National Institute of Health. The program included 11 pre and 3 post doctoral trainees.

PROBLEMS OF OUTER SPACE BEING INVESTIGATED BY OTHERS ON THE CAMPUS

Dr. Ichiye has prepared models of oceans, Fig. 10, in the same laboratory in which Dr. Hess is erecting atmospheric models. These two lines of research provide cross-fertilization of interests.

Research by Dr. Sherwood Reichard on antiradiation extracts from the rat has been supported in its initial stages by the Institute.

Professor Colin H. Barrow has established a radio observatory close to the campus and has begun a program of observations of Jupiter, Fig. 11. Polarized radio bursts have been recorded.

GUEST SPEAKERS AND VISITORS

The guest speakers and visitors listed below were invited during the year from out of town to participate in space related activities.

Dr. Philip Abelson
Dr. J. J. Wolken
Dr. R. H. Knapp
Dr. F. Haurowitz
Dr. D. Pitelka
Dr. H. N. Christensen
Dr. J. Gowen
Dr. F. S. Sisler
Dr. Ian Kaplan
Dr. Dale Jenkins

Others who visited were:

Dr. Rusty Mann
Dr. G. E. Fogg
Dr. Webb Haymaker
Dr. Robley Light
Dr. Julius Marmur
Dr. David B. Tyler
Dr. D. I. Packham
Dr. George Wald
Dr. Leroy Augenstine
Dr. Robert Allen

LECTURES AND PARTICIPATION IN CONFERENCES AND SYMPOSIA

S. W. Fox

Participation with Dr. Philip Abelson in a two-man symposium on Chemical Origins of Life, before the Washington, D. C. section of the American Chemical Society, 9 November 1961.

Lecture on Biochemical Origins, at University of California at Santa Barbara, 14 November 1961.

Paper on Borders of Evolution of Protein, in symposium on Extraterrestrial Biochemistry and Biology, AAAS national meeting, Denver, 27 December 1961.

Address on A Chemical Model of Spontaneous Generation, Sigma Xi, University of Florida, 22 January 1962.

Lecture on Proteinoid Microspheres, National Institutes of Health, 27 April 1962.

Paper on The Origin of Protein and Formed Microparticles, in New York Academy of Sciences conference on The Problems of Environmental Control of the Morphology of Fossil and Recent Protobionta, New York City, 30 April 1962.

Lecture on Experiments Related to the Origin of Life, Stanford University, 3 May 1962.

Participation in Space Science Board Summer Study on space science, Iowa City, in July 1962 on Space Biology, in August 1962 on sterilization of space vehicles.

Collection of volcanic samples at Stromboli, 3 - 5 September 1962.

Lecture on Thermal Polyamino Acids, Cornell University Medical College, 15 October 1962.

Principal address on Experiments Suggesting Origins of Amino Acids and Proteins in University of Illinois symposium honoring W. C. Rose, T. Hamilton and H. H. Mitchell, 17 October 1962.

The above lectures and participations represent those commitments

which could be accepted on the basis that they were spaced sufficiently, and usually coordinated with other fruitful trips such as visits to the Ames Research Center.

S. Hess

University of California at Santa Barbara conference, 11 April 1962.

Jet Propulsion Laboratory, Pasadena, California, conferences on rocket vehicles to Venus and Mars, 12 April 1962.

Institute for Space Sciences, New York, N. Y. for an invitational conference on the planet Jupiter, 1 - 17 October 1962.

NASA-University Conference on the Science and Technology of Space Exploration, Chicago, 1 - 3 November 1962.

C. B. Metz

Served as Chairman of Training Program in Fertilization and Gamete Physiology Program, Woods Hole, Massachusetts, 12 June through 25 August 1962.

Lecture to Training Program members and scientific community at Woods Hole, Immunological Studies on Fertilization, 3 July 1962.

Speech before Conference on Immuno-Reproduction, La Jolla, California, Immunochemical Studies on Fertilization Mechanisms in Sea Urchins, 10 September 1962.

Immunochemical Studies on Fertilization, Department of Anatomy, Tulane University Medical School, 7 March 1962.

Papers presented at the March 1962 meeting of Southeastern Region Developmental Biology Conference, Tulane University, and American Society of Zoologists meeting in conjunction with AIBS August 1962.

Lecture on fertilization, N.S.F. High School Summer Institute, Northeastern University, 19 July 1962.

Two in-house lectures at NASA headquarters, The Cell as a Biological Unit, 3 April 1962 and Cell Division, 1 May 1962.

Served as ad hoc member of cell biology study section, Research Grants Division, N.I.H., April 1962.

Served on Research Grants Committee, Florida Division, American Cancer Society.

RECOGNITIONS

United States Science Exhibit Medal, 1962, Department of Commerce to S. W. Fox.

Preparation of high school teaching film on Origin of Life for AIBS by S. W. Fox.

Both Drs. Fox and Hess were invited to serve in the Space Science Board Summer study at Iowa City.

Participation in Voice of America Forum Lecture Series: Arts and Sciences in mid-century America by C. B. Metz.

During the year both Drs. Hess and Metz were listed in Who's Who in the U. S. A., a volume which now includes all three of the faculty members of the Institute.

PUBLICATIONS FROM INSTITUTE AND ANTECEDENT INVESTIGATIONS

Publications of S. W. Fox

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With Joseph E. Johnson and Mavis Middlebrook, "Pyrosynthesis of Aspartic Acid and Alanine from Citric Acid Cycle Intermediates." J. Am. Chem. Soc. 77, 1048 (1955).

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With Kaoru Harada and Allen Vegotsky, "Thermal Polymerization of Amino Acids and a Theory of Biochemical Origins." Experientia 15, 81 (1959).

With Kaoru Harada and Jean Kendrick, "Production of Spherules from Synthetic Proteinoids and Hot Water." Science 129, 1221 (1959).

"A Chemical Theory of Spontaneous Generation." In The Origin of Life on the Earth. London, Pergamon Press, 256 (1959).

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Scientific Reviews and Books

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Publications of Seymour L. Hess

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The publications listed do not present all of the publications of each of the senior members of the Institute. They are publications of the three permanent faculty members on research deemed to be within the present scope of the research of the Institute.

The numerous publications of Tadao Hayakawa, Cecilia Lutwak-Mann, Thaddeus Mann, and Margaret Menzel are also not included. Also not included are some pertinent publications of Kaoru Harada.

The publications of Thaddeus Mann are represented here, however, by reference to his monograph on The Biochemistry of Semen (Methuen and Co., ltd, London, 1954) and its extensive bibliography.

Pertinent publications of the investigators memtioned are scheduled for inclusion in a subsequent report. When this listing is reported, that report and this will constitute a total bibliography of space bioscience articles within the two issues, for the Institute.

ACKNOWLEDGMENT

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